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Title 1: Tri-octahedral bentonites as potential technological feed additive for *Fusarium* mycotoxin reduction.

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Abstract

In 2009 the EU Regulation 386/2009 established a new functional group of feed additives called "substances for reduction of the contamination of feed by mycotoxin" (EC 386/2009). Later, a di-octahedral bentonite (1m558) was authorized, as an anti-aflatoxin additive, being the only additive of this group authorized to date. This work aims to demonstrate the effectiveness of other bentonites, such as tri-octahedral bentonites, versus *Fusarium*-mycotoxins, since very few adsorbents have proved their effectiveness versus this group of mycotoxins. For this purpose, 7 bentonites (six of them tri-octahedral) and 7 commercial adsorbents, added at 0.02% (w/v), were assayed in an *in vitro* adsorption experiment using two simulated gastro-intestinal (GI) juices (pH 1.3 and 6.8) versus zearalenone (ZEN: 0.1-5 mg/L), fumonisin B_1 (FB_1: 1-10 mg/L) and deoxynivalenol (DON: 2-10 mg/L). Mycotoxin adsorption data were fitted to Langmuir and Freundlich isotherms. *In vitro* adsorption experiments showed that, ZEN and FB_1 (in the latter case only in acid medium) were partially adsorbed, while the adsorption of DON was negligible. Moreover, the increase of adsorbent dose (up to 0.20%, w/v) significantly improved the *in vitro* adsorption of ZEN and FB_1, reaching >90% of adsorption. The present work proposes the use of some tri-octahedral bentonites as feed additives for *Fusarium*-mycotoxin reduction.

Keywords: Tri-octahedral bentonite; *Fusarium* mycotoxins; Animal feed; Decontamination; Adsorption isotherms.

Introduction

Fungal contamination of grains is a worldwide problem, especially because cereals used for livestock feed are often imported and exported all over the world (Zulkifli and Zakaria 2017). Cereals, especially maize and wheat, constitute most of the daily diet of animals, and are important ingredients in animal compound feeds (Pinotti et al. 2016). *Fusarium* mycotoxins such as fumonisins (FBs), trichothecenes (with a special relevance deoxynivalenol, (DON)) and zearalenone (ZEN)) are present on a worldwide basis in cereal grains (mainly in maize, wheat, and barley), animal feeds and forages, and can cause a variety of diseases in a wide range of animal species. Among fusariotoxins, FBs are a group of major importance, and fumonisin B_1 (FB_1) is the most toxic among FBs, which represents about 75% of total FBs synthesized by *Fusarium* species (Galeana-Sánchez et al. 2017). Acute and chronic toxicity by FB_1 has been largely reported in several animal species, including carcinogenicity and cardiovascular toxic effects (Gelderblom et al. 1991; Pierron et al. 2016). Indeed, FB_1 is currently classified in group 2B, as possibly carcinogenic to humans, by the International Agency for Research on Cancer (IARC, 2002). On the other hand, the toxicity of ZEN has been associated with estrogenic effects and reproductive disorders in animals and, possibly, in humans (Wang et al. 2012), while DON is a known mycotoxin produced mainly by the plant pathogens *Fusarium graminearum* and *F. culmorum*. It is one of the major trichothecenes found in corn and small-grain cereals. It causes weight loss, anorexia and decreased nutritional efficiency, mainly in pigs (Pierron et al. 2016). Although DON is not as toxic as other mycotoxins (it is not classifiable as to its carcinogenicity to humans by IARC), it is one of the most common contaminants of cereals worldwide and it is responsible of substantial economic impact, mainly in the major affected products, such as wheat, barley, oat and rye (Marin et al. 2013; Femenias et al. 2020).

The high occurrence of *Fusarium* toxins in food and feed products, and the risks associated with *Fusarium* exposure have been widely reported (Streit et al. 2012; Feijó Corrêa et al. 2018). Single species may produce more than one mycotoxin concomitantly, and different *Fusarium* species may proliferate in the same plant, both leading to the co-occurrence of two or more mycotoxins (Feijó Corrêa et al. 2018). Moreover, *Fusarium* mycotoxins frequently coexist with other mycotoxins as aflatoxin B_1 (AFB_1) and ochratoxin A (OTA) (Ibáñez-Vea et al. 2011; Robinson et al. 2012; Iqbal et al. 2014). The major problem associated with mycotoxin contamination of feed is not the acute disease episodes but the low level of continuous mycotoxin ingestion, which causes an array of metabolic disturbances resulting in poor animal productivity (Bryden, 2012; Feijó Corrêa et al. 2018). In fact, farm animals often show symptoms of chronic mycotoxicoses, even when the concentrations of individual mycotoxins do not exceed permitted or recommended levels (EC, 2006; Grenier and Oswald, 2011; Streit et al. 2012; Wielogórska et al. 2016).

An interesting approach for detoxification of feedstuffs is the use of clay materials, such as bentonites, that can bind mycotoxins in the gastrointestinal (GI) tract, thus reducing the extent of their absorption and systemic toxicity. Bentonites have been considered as promising adsorbents for high-efficient removal of mycotoxins from animal feeds because they are eco-friendly, low-cost, and highly efficient in adsorption of mycotoxins (Phillips et al. 2008, 2019; Robinson et al. 2012; Li et al. 2018; Wang et al. 2020). However, most of the studies of mycotoxin adsorption using bentonites appear to focus only on a limited group of mycotoxins (mainly polar aflatoxins (AFs) such as AFB_1), and shows nearly no affinity for non/weak polar mycotoxins such as OTA, ZEN and DON (Greco et al. 2019). In fact, most of the commercially available mycotoxin-adsorbents failed in sequestering *in vitro Fusarium* mycotoxins (Ramos et al. 1996a; Ramos et al. 1996b; Avantaggiato et al. 2007; Avantaggiato et al. 2014; Vila-Donat et al. 2018; Wang et al. 2012).

To date, only a di-octahedral bentonite (1m558) has been authorized as technological additive for reduction of the contamination of feed for ruminants, poultry and pigs by mycotoxins (AFB_1) (EC, 2013). Therefore, feed additives with detoxification functions to different group of mycotoxins are sought.

The present study was designed to evaluate *in vitro* adsorption percentages and equilibrium adsorption isotherms of seven bentonites (mainly tri-octahedral bentonites that previously showed *in vitro* high adsorption of AFB_1 and OTA) (Vila-Donat et al. 2019), and seven commercial adsorbents versus the three most important fusariotoxins (FB_1, ZEN and DON). Finally, to determine the effect of increasing the dosage of the two most effective tri-octahedral bentonites, in their adsorption capacity.

Materials and methods

Reagent and materials

All chemicals used were of HPLC grade. Methanol, acetonitrile and acetic acid were purchased from Scharlab S.L (Barcelona, Spain). Ultrapure water was produced by a Milli-Q® system at 22 µm (Millipore, Bedford, MA, USA). For the adsorption experiments with DON and ZEN, simulated gastric and intestinal juices were prepared according to the United States Pharmacopeia/the National Formulary (USP23/NF18). Simulated gastric juice was prepared by adding 2 g of sodium chloride (Fischer Scientific, Loughborough, UK), 3.2 g of pepsin from porcine gastric mucosa with ≥400 units/mg protein (Sigma-Aldrich, St. Louis, MO, USA), and 7 mL of hydrochloric acid (37%) (Panreac, Barcelona, Spain) to 1 L of water (pH 1.3). Intestinal juice was prepared by adding 6.8 g of potassium dihydrogen phosphate (Panreac, Barcelona, Spain), 0.2 mol/L sodium hydroxide, 1.25 g of pancreatin from porcine pancreas 8 x USP specifications (Sigma-Aldrich, St. Louis, MO, USA) and 6.75 g of lactose (Probus, S.A, Badalona, Spain) to 1 L of water (pH 6.8). For the adsorption experiments with FB_1, acid and

basic buffers (at pH 1.3 and 6.8) were used instead of GI juices in order to avoid interferences in chromatograms due to the reaction of the pre-column FB_1 derivatizing agent with the components of the juices. Acid buffer (pH 1.3) was prepared by adding hydrochloric acid (0.1 mol/L) and potassium chloride (0.1 mol/L) to 1 L of water. Basic buffer (pH 6.8) was prepared by adding sodium phosphate monobasic (0.1 mol/L) and sodium phosphate dibasic (0.1 mol/L) to 1 L of water. For the adsorption experiments at pH 5, the test medium was acetate buffer. It was prepared by dissolving 0.1 mol/L acetic acid (Panreac, Barcelona, Spain) and 0.1 mol/L sodium acetate (Sigma-Aldrich, St. Louis, MO, USA) to 1 L of water. OPA solution for FB1 derivatization was prepared by adding 40 mg of *o*-phthaldialdehyde (OPA) (Merck KGaA, Darmstadt, Germany) to 1 mL of methanol, 5 mL of 0.1 mol/L disodium tetraborate decahydrate (Panreac, Barcelona, Spain) and 50 μL of 2-mercaptoethanol (Scharlab S.L, Barcelona, Spain).

Analytical standards

Mycotoxin standards of DON, FB_1 and ZEN were supplied by Biopure Romer Labs Diagnostic Gmbh (Tulln, Austria). Declared purity of all standards was in the range of 97.6 to 99.5%. Stock solutions (1 mg/mL) were prepared by dissolving 5 mg of dried mycotoxin in 5 mL of acetonitrile (HPLC grade). These solutions were properly diluted with gastric or intestinal simulated juices, or buffers (in the case of FB_1) to prepare the mycotoxin working solutions for adsorption experiments. Standard solutions were prepared in the mobile phase for HPLC calibration. Stock solutions were stored in amber vials at -20 °C and brought to room temperature before use.

Samples

Seven bentonites (B1 to B7, six of them tri-octahedral bentonite), kindly provided by a mining company, and selected by their proved high AFB1 and OTA adsorbing capacities (Vila-Donat et al. 2019), were studied along with seven commercially available adsorbents, mainly

144 di-octahedral bentonites (C1 to C7) (Table 1) at 0.02 % (w/v). Later, two tri-octahedral
145 bentonites (B2 and B4) were tested at doses up to 0.20 % (w/v).

146 ***Equilibrium adsorption isotherms***

147 The fourteen adsorbents previously described were subjected to an *in vitro* equilibrium
148 adsorption experiment, by testing a fixed amount of adsorbent (0.02% w/v) against six
149 concentrations of ZEN (0.10, 0.50, 1, 2, 3 and 5 mg/L), FB_1 (1, 2, 4, 6, 8, and 10 mg/L) and
150 DON (2, 4, 6, 8, 10 and 12 mg/L), separately, using two simulated physiological GI juices or
151 buffers (at pH 1.3, and at pH 6.8) at constant temperature (37 ºC).

152 To this purpose, a suspension of clay was prepared by weighing 0.1 g of each adsorbent
153 into a flask with 10 mL of H_2O and shaking it on a magnetic stirrer. While stirring, 80 µL of
154 the suspension were pipetted, to make the clay concentration 0.02% (w/v), into 10 mL screw
155 cap Falcon polypropylene tubes to which were added next 4 mL of gastric (at pH 1.3) or
156 intestinal juice (at pH 6.8) along with ZEN, FB_1 and DON.

157 All tubes were placed horizontally in an Orbital Shaker-Incubator (4g, 37 ºC, 2h). After the
158 incubation period, the adsorbent materials were separated by centrifugation (8800g, 4 ºC, 10
159 min). The supernatants were transferred to clean vials and analysed for the residual mycotoxin
160 content by HPLC-FD/DAD for ZEN, FB_1, and DON, as reported below. Experiments were
161 carried out in triplicate by HPLC analysis. Positive controls (also in triplicate) were prepared
162 by adding 4 mL of gastric/intestinal juices with mycotoxins and without the addition of
163 adsorbent.

164 ***Data calculation and curve fitting***

165 The amount of adsorbed mycotoxin per unit of mass of adsorbent (Q_{eq}), was calculated
166 using the equation 1 described in Vila-Donat et al. (2019).

167 Adsorption equilibrium is established when the quantity of the mycotoxin being adsorbed
168 (Q_{eq}) is equal to the quantity being desorbed. Then the equilibrium concentration in solution

(C_{eq}) remains constant. Adsorption isotherms were obtained by plotting the amount of adsorbed mycotoxin (Q_{eq}) per unit of mass of adsorbent (mg toxin/g adsorbent) against the equilibrium non-adsorbed toxin concentration (mg/L) (C_{eq}). Among the theoretical adsorption models reported in literature, that describe the equilibrium relationship between adsorbed and non-adsorbed amounts, Langmuir and Freundlich models provide the best description of mycotoxin adsorption (Vila-Donat et al., 2019). The Langmuir model (Langmuir, 1916) is valid for monolayer adsorption to a surface with a finite number of identical sites, while, the empirical Freundlich (Freundlich, 1906) equation, is based on adsorption onto a heterogeneous surface.

In the present work, Langmuir and Freundlich models were tested. The equations used for calculations were described in Vila-Donat et al. (2019) (eq. 2 and 3, respectively).

Both adsorption isotherms were obtained by plotting the concentration of ZEN/FB_1 in solution after equilibrium (C_{eq}) against the amount of ZEN/FB_1 adsorbed per unit of weight of each adsorbent (Q_{eq}). Data obtained from the equilibrium adsorption experiment were transferred to the statistical program JMP Pro 14.1.0 and both isotherm models fitted. The isotherm parameters were estimated by non-linear regression. Goodness of fit was assessed and the parameters involved in the adsorption mechanism (maximum adsorption capacity and adsorption affinity) were estimated (Tables 5 and 6).

HPLC conditions

Analysis were performed on the HPLC-FD equipment described in our earlier work (subsection 2.7) (Vila-Donat et al., 2019) to which the diode array detector DAD HS (G7117C) was also coupled. ZEN and FB1 were analysed by using liquid chromatography with fluorescent detection (HPLC-FD), while DON was analysed by using liquid chromatography with diode array detector (HPLC-DAD).

The chromatographic separation of these compounds (ZEN, FB1 and DON) was achieved on a Kinetex PFP 100 Å 5 µm, 4.6 x 150 mm column (Phenomenex®, California, USA), and the

column temperature was 40 ºC. For ZEN, the flow rate was 1 mL/min using the following mobile phase: A: acetonitrile, B: methanol, C: acetic acid 0.1 % in gradient mode. The gradient elution program started with an initial elution at 15% A, 0% B, 85% C for 5 min, which changed to 14% A, 27% B, 59% C until 7 min, and continued 90% A, 0% B, 10% C until 12 min when the elution changed back to the initial 15% A, 0% B, 85% C for re-equilibration of the column. The injection volume was 100 µL. Excitation and emission wavelengths were λ_{ex} = 276 nm and λ_{em} = 460 nm.

For FB_1 analysis a pre-column derivatization with OPA was needed since this mycotoxin lacks of chromophores and is not naturally fluorescent. 25 µL of the OPA mixture previously prepared was automatically mixed with 25 µL of each sample before the injection by setting the sample injector program. The flow rate was 1.2 mL/min using the following mobile phase: A: acetonitrile, B: methanol, C: acetic acid 0.1% in gradient mode. The gradient elution program started with an initial elution at 15% A, 0% B, 85% C for 10 min, which changed to 5% A, 61% B, 34% C until 16 min, and to 5% A, 72% B, 23% C until 20 min when the elution changed back to the initial 15% A, 0% B, 85% C for re-equilibration of the column. The injection volume was 100 µL. Excitation and emission wavelengths were λ_{ex} = 335 nm and λ_{em} = 460 nm.

For DON, the flow rate was 0.6 mL/min using the following mobile phase: A: water, B: acetonitrile in gradient mode. The gradient elution program started with an initial elution at 99% A, 1% B, for 7 min, which changed to 90% A, 10% B until 14 min, when the elution changed back to the initial 99% A, 1 % B for re-equilibration of the column. The injection volume was 50 µL. Two different wavelengths (220 nm/360 nm) were employed to monitor and quantify the targeted compounds.

Method validation

Quantification of mycotoxins was based on the external standard method using calibration curves fitted by linear regression analysis. The linearity of the calibration graphs was studied by injecting seven-points calibration curves at concentrations of 0.08-5 mg/L, 0.15-10 mg/L and 0.08-10 mg/L for ZEN, DON and FB_1, respectively. Correlation coefficients obtained were ≥0.9999, ≥0.9998 and ≥0.9995 for ZEN, DON and FB_1, respectively. Regarding the sensitivity, the limits of detection (LOD) of the method for all mycotoxins were assessed. LOD was calculated to be 0.04 mg/L, 0.1 mg/L and 0.08 mg/L for ZEN, DON and FB_1 respectively. The repeatability and reproducibility of the method was assessed by injecting five times standard on the same day (intra-day) and over 5 days (inter-day), respectively.

Recoveries were calculated taking into account the blank tubes (positive controls) which contained mycotoxin and did not contain adsorbent. Once finished GI digestion, tubes were centrifuged and separated in two phases: the precipitate and the supernatants. Supernatants or final solutions were filtered and directly analysed for the residual mycotoxin content by HPLC. The amount of adsorbed mycotoxin was calculated as the difference between the amount of mycotoxin in the supernatant of the positive controls and the amount found in the supernatant of the experimental tubes. This amount was then expressed as a percentage of the positive controls.

Statistical analysis

Fitting of adsorption isotherms and estimation of suitable parameters was carried out using JMP Pro 14.1.0.

Results

Adsorption experiments

Six mycotoxin concentrations (at levels according to the EU recommendations for the studied *Fusarium* mycotoxins in animal feed) were assayed to measure mycotoxin adsorption

in simulated GI juices (at pH 1.3 and at pH 6.8), where a known amount of mycotoxin reacted with a known amount of adsorbent (0.02% w/v).

From results presented in Table 2, it can be seen that ZEN adsorption was very low when high levels of mycotoxin (1-4 mg ZEN/L) were tested. However, at the lower levels tested (0.1-0.5 mg ZEN/L) the adsorption increased, being the adsorption percentages slightly higher in the intestinal than in the gastric simulated juice. The tri-octahedral bentonites (B1-B7) tested showed adsorption percentages ranging from 30.6-70.4% in the intestinal juice at the lower dose of toxin assayed (0.1 mg ZEN/L). In the same conditions, only three commercial products (C1, C4, and C5) achieved similar adsorption.

Concerning FB_1 adsorption, the results showed that it was extremely affected by pH. In acid medium (pH 1.3) adsorbents were very effective in binding FB_1. In fact, adsorption percentages ranged from 36.7 to 90.6% at the higher level tested (10 mg FB_1/L) and from 72.5 to >97.6% at the lower level tested (1 mg FB_1/L). Again, tri-octahedral bentonites showed, generally, higher adsorption percentages than commercial products. Only two commercial products (C1 and C5) achieved similar adsorption at the lower level of mycotoxin tested (1 mg/L) (Table 3). However, when the equilibrium adsorption experiment was performed at pH 6.8, adsorption became negligible for all adsorbents (Table 3).

Regarding DON, as expected, assayed bentonites did not show good adsorption capacities. In fact, all adsorbents evaluated, including the commercial ones, showed poor affinity towards DON, and did not exceed, at best, 22% adsorption (B2) (Table 4).

Adsorption isotherms

With regard to ZEN adsorption, the maximum adsorbed amounts (Q_{max}), derived from Langmuir isotherms at pH 1.3 and at pH 6.8, are summarized in Table 5. Comparing the results obtained with the 14 adsorbents at pH 6.8, the maximum adsorption capacity was higher for adsorbents B6, B7, and B4, as the calculated values were 5.69±0.8, 3.70±0.9, and 3.54±0.9

mg/g, respectively (Table 5). According to the K_L constant, which is related to the affinity of the adsorbent, B2 sorbent at pH 6.8 showed a greater adsorption affinity for ZEN. Also, the K_F Freundlich constant related to adsorption capacity of the adsorbent, showed that bentonites B6, B4 and B7 had the higher adsorption capacities (at pH 6.8) (Table 5). According to the data obtained by plotting ZEN adsorption isotherms, comparable or even better adsorption capacities were obtained with tri-octahedral bentonites (B1- B7) than with di-octahedral bentonites (C1-C7).

Regarding FB_1 adsorption isotherms, the fits depended on the pH of the medium. According to Langmuir model, the maximum adsorption capacity (Q_{max}) at pH 1.3 was 47.9±13.6, 41.6±5.4 and 37.9±6.3 mg/g for bentonites B6, B2 and B5, respectively (Table 6), while at pH 6.8 the model did not fit. On the other hand, the K_F Freundlich constant showed adsorbents B6, B5, B1 and B2 as the adsorbents with the higher adsorption capacity, with values ranging from 26.8±1.2 to 43.8±9.1 mg/L (at pH 1.3) (Table 6). Generally, FB_1 adsorption data were better fitted by Freundlich than by Langmuir equation taking into account that in many cases Langmuir model did not converge, as can be observed in Table 6. Examples of the adsorption curves obtained are presented in Fig. 1 and 2.

Effect of the adsorbent dosage

The effect of the adsorbent dose on the mycotoxin adsorption was studied with the two best tri-octahedral bentonites assayed (B2 and B4) by increasing the dose from 0.02% to 0.12 and 0.20% (w/v), in acetate buffer at pH 5**.** Concentrations of mycotoxin tested were 0.5 mg/L, 6 mg/L and 2 mg/L for ZEN, FB_1 and DON, respectively. Results showed that DON adsorption did not seem to be affected by these increases, since the adsorption percentage was negligible at all doses tested (Fig. 3). Surprisingly, for ZEN, and FB_1, the percentage of mycotoxins removed from buffer increased with increasing dosages of bentonite (Fig. 3). Experimental values for the adsorption of ZEN and FB_1 were in the ranges of 13.7- 60.6% and 49.2- 95.1%

for adsorbent B2, and 38.2- 94.1% and 54.4- 97.9% for adsorbent B4, respectively. In both cases, ZEN and FB_1 adsorption improved considerably (almost doubled) when the dose of adsorbent increased from 0.02% to 0.12%, and adsorption also improved when dose increased from 0.12 to 0.20%, although not so clearly (Fig. 3). The highest adsorption of ZEN and FB_1 (94.1% and 97.9%, respectively) was achieved with the tri-octahedral bentonite B4 at 0.20% (w/v) (Fig. 3).

Discussion

In the present study fourteen adsorbents (mainly tri-octahedral bentonites and di-octahedral bentonites) were assayed to measure *Fusarium* mycotoxin (ZEN, FB_1 and DON) adsorption in simulated GI juices (at pH 1.3 and 6.8), where a known amount of mycotoxin reacted with a known amount of adsorbent (0.02% w/v). The selected pH values were 1.3 and 6.8 for gastric and intestinal simulation of the porcine GI tract, respectively, since pigs are the most sensitive to fusariotoxins and during digestion, the pH of the food bolus is not constant. In fact, in monogastric animals, there is a large change in the pH along the GI tract; it can vary from 1.2 to 4.5 in stomach, increasing from 5 to 7.5 in the intestinal lumen (Greco et al. 2019).

Bentonite are phyllosilicates characterised by a sheet structure made of layers of polyhedra of silicon oxide with tetrahedral coordination between which there is an octahedral layer. The octahedral layers contain atoms of aluminium, iron (II or III) or magnesium in their interior. (Hurlbut and Klein, 1982). The structural differences among both bentonites were previously described in our recently published article (Vila-Donat et al. 2019). It can be noted that tri-octahedral bentonites (saponite), characteristically have lower Al_2O_3 and, higher MgO contents, than di- octahedral (montmorillonite). The di-octahedral bentonite is one of the most abundant, and most of the adsorption experiments have been performed with this type of bentonite. As far as we know, this is the first work in which the efficacy of tri-octahedral bentonites *versus* fusariotoxins has been evaluated.

318 It should be noted that the bentonites of the study (B1-B7) are composed only by the
319 mineral fraction (mainly tri-octahedral bentonite), while the commercial products assayed (C1-
320 C7) contain, apart from the mineral fraction (mainly di-octahedral bentonite), other components
321 such as organic additives which theoretically increase their effectiveness or extend its spectrum
322 of action against *Fusarium* mycotoxins (Table 1).

323 According to the provisions set by Regulation (EU) No 1060/2013, the only additive
324 approved to date demonstrated its effectiveness when tested at 0.02% (w/v). Unlike many other
325 already published works, in this work, tests were performed using this adsorbent dosage. In the
326 same way, the levels of mycotoxins assayed were at levels according to the EU
327 recommendations for the studied *Fusarium* mycotoxins in animal feed which, at present, ranged
328 for DON from 0.9 to 12 mg/Kg, for ZEN from 0.1 to 3 mg/Kg, and for FB_1 from 5 to 60 mg/Kg,
329 depending on the animal species (EC, 2006).

330 The results presented in this work show that tri-octahedral bentonites were able to adsorb
331 two fusariotoxins (ZEN and FB_1) to some extent, while these bentonites failed adsorbing DON.
332 Concerning FB_1 adsorption, the results showed that adsorbents were very effective at all
333 concentrations in binding FB_1, but only in acid medium (pH 1.3). Taking into account that FB_1
334 adsorption decreases dramatically at basic pH, it can be supposed that adsorption could be
335 affected in the intestinal tract of monogastric animals and desorption could happen. In fact, FB_1
336 is an aliphatic long-chain molecule, with four carboxylic groups, the pKa of which is around 5
337 and strong pH effects are therefore expected (Boudergue et al. 2009). This fact has been
338 previously described with OTA by our research group (Vila-Donat et al. 2019), showing that
339 OTA adsorption by different bentonites was higher when this mycotoxin was tested in gastric
340 juice (pH 1.3), and probably because the adsorption is propitious when the molecule was
341 uncharged. Similarly to OTA, different mechanisms may be involved in FB_1 adsorption
342 depending on pH and the degree of ionization of molecules. It is known that adsorption of

343 mycotoxins by adsorbents depends not only on the mycotoxin but also on the adsorbent
344 properties like polarity, size, and solubility. Medium pH can affect the surface charge of
345 adsorbents as well as the degree of ionization of mycotoxins, and subsequently it can lead to a
346 shift in reaction kinetics and equilibrium characteristics of the adsorption process.

347 Regarding ZEN, adsorption was higher at the lower level of toxin assayed (0.1 mg ZEN/L),
348 and in the intestinal juice, suggesting that no desorption should happen during transit through
349 the GI tract. ZEN is a macrocyclic molecule. The presence of a diphenolic group makes it a
350 weak acid. Due to the relatively high value of the pKa, pH effects should be much weaker than
351 for fumonisins (Boudergue et al. 2009). In this case, tri-octahedral bentonite would fulfill the
352 condition that an efficient adsorbent should retain the adsorbed mycotoxin during the transit
353 through the different GI compartments. On the other hand, results obtained with tri-octahedral
354 bentonites were comparable to three commercial products (C1, C4, and C5) that contained
355 mainly the di-octahedral bentonite 1m558, the only additive of this group authorized to date as
356 adsorbent of AFs (EC 1060/2013) (Table 1).

357 Regarding DON, our results are in agreement with other previous works in which several
358 adsorbent materials, including smectites, activated carbon (AC), polymers such as
359 cholestyramine and other commercial products (composed of mixtures of clays and organic
360 compounds such as yeast-cell walls and plant extracts) were tested, and no adsorbent materials,
361 with the exception of AC, and cholestyramine showed relevant ability in binding DON (Ramos
362 et al. 1996 a,b; Avantaggiato et al. 2003, 2005; Sabater-Vilar et al. 2007). About AC, the DON
363 adsorption abilities vary widely depending on the type of carbonaceous substances and
364 activation processes. Moreover, the application of AC in animal feed could adsorb minerals,
365 vitamins and other nutrients as well, and the effectiveness of AC towards DON could not be
366 confirmed *in vivo* (Sabater-Vilar et al. 2007; Avantaggiato et al. 2004). With respect to

cholestyramine, the high cost of this polymer would be a limiting factor for its practical implementation (Ramos et al. 1996 b; Avantaggiato et al. 2005).

In the mentioned studies, authors concluded that the smectite clays commonly used in animal feed to detoxify mycotoxins were effective with respect to AFs but failed in sequestering DON. This could be due because DON is a hydrofilic, non-ionisable molecule, with a bulky epoxy group, which does not favour adsorption to plane surfaces. As a consequence, DON is very few adsorbed by bentonites and by other planar adsorbing agents, probably due to the lack of structural complementation (Boudergue et al. 2009; Wielogórska et al. 2016). Conversely, planar molecules, such as AFB_1, are well adsorbed by planar adsorbing agents such as bentonite. In fact, results from the latest and novel works with bentonites and AFB_1 have indicated that these clays possess active sites within their interlamellar regions for aflatoxin adsorption, and the carbonyl moiety in the aflatoxin molecule is important for binding (Wang et al. 2020).

In order to investigate the mechanism of mycotoxin adsorption (ZEN and FB_1) by different adsorbents, both adsorption isotherms (Langmuir and Freundlich) were fitted at pH 1.3 and at pH 6.8. Regarding DON, it was not possible fit the data to the models given the low adsorption capacity. Generally, both adsorption models (Langmuir and Freundlich) provided a good fit to experimental adsorption data of ZEN (Fig. 1). In order to evaluate the fit of the isotherm to the experimental data, the optimization procedures require a statistical goodness-of-fit-measure. In this study the Residual Root Mean Square Error (RMSE) was employed to determine which isotherm better fitted to our results. Taking into account RMSE values obtained, Freundlich model showed a slightly better fit than the Langmuir isotherm (Table 5). Differently, Avantaggiato et al. (2014) tested the adsorption efficacy of a biosorbent such as grape pomace against different mycotoxins, and Langmuir equation was found to be the best fitting isotherm model for ZEN adsorption by this organic sorbent.

As previously mentioned, the Langmuir model is valid for monolayer adsorption onto a surface with a finite number of identical sites. In fact, Langmuir equation includes a maximum adsorption capacity parameter in its formula that represents the maximum adsorbed amount of mycotoxin to form a complete monolayer on the adsorbent surface, corresponding to saturation sites (Vila-Donat et al. 2019). In contrast to the Langmuir model, Freundlich model is based on adsorption onto a heterogeneous surface. In fact, the Freundlich equation gives information on the heterogeneity of the binding sites and the adsorption intensity (Vila-Donat et al. 2019). The fact that the Freundich isotherm fits better the experimental data may be due to the relatively heterogenous binding sites of bentonite surface, since the Freundich equation assumes that the adsorbent surface has multilayer adsorption behaviour.

In the same way, FB_1 adsorption data were better fitted by Freundlich than by Langmuir equation, taking into account that in many cases Langmuir model did not converge, as can be observed in Table 6, suggesting a heterogeneous binding mechanism. In contrast, FB_1 adsorption isotherms on AC fitted better to the Langmuir model, which indicated that saturation was reached (Avantaggiato et al. 2005).

Finally, the effect of dosage on mycotoxin adsorption of B2 and B4 bentonites was studied to calculate the optimal adsorbent dosage for further experiments. A 10-fold dose increase of bentonite B4 (0.20% w/v) improved the adsorption of ZEN and FB_1 reaching 94.1% and 97.9%, respectively (Fig. 3). It should be noted that this bentonite B4 when previously tested at 0.02% (w/v) versus ZEN did not exceed 40% of adsorption (Table 2). These results were in accordance to our previous work in which the percentage of OTA adsorbed increased proportionally with increasing dosages of tri-octahedral bentonites (Vila-Donat et al. 2019).

To conclude, feed additives with detoxification functions versus different group of mycotoxins are highly demanded by the feed industries. Tri-octahedral bentonites could offer technological applications as feed additives to reduce structurally different mycotoxins, by

inducing the formation of a mycotoxin-bentonite complex which would avoid the GI absorption of these toxic metabolites. However the efficacy of adsorbents depends on the type of mycotoxin, the dose of mycotoxin and adsorbent tested, as well as the pH of the medium in which *in vitro* tests are perfomed.

In vitro tests are considered as a screening tool for studying the potential of substances to act as mycotoxin binders. However, the *in vitro* experiments conducted should be taken in account with caution and should be confirmed by *in vivo* studies, such as the works carried out by Robinson et al. (2012), Pollock et al. (2016) and Phillips et al. (2019), attesting that the adsorbents maintain their adsorptive capacity in the conditions of the real GI tract, and they do not have any collateral negative effect, as, for example, the adsorption of essential nutrients or vitamins.

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Declaration of interest statement

The authors declare there are no conflicts of interest.

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563 **Figure captions**

564 **Fig. 1** Langmuir isotherms obtained at 37 ºC in gastric (on the left, at pH 1.3) and intestinal (on
565 the right, at pH 6.8) simulated juices by testing a fixed amount of adsorbent B4 (0.02%, w/v)
566 versus increasing concentrations of ZEN (0.1-5 mg/L).

567 **Fig. 2** Freundlich isotherms obtained at 37 ºC in acid buffer (at pH 1.3) by testing a fixed amount
568 of adsorbent B2 (left) and B4 (right) (0.02%, w/v) versus increasing concentrations of FB_1 (1-
569 10 mg/L).

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571 **Fig. 3** Effect of B2 (on the left) and B4 (on the right) adsorbent dosage on FB_1, ZEN, and DON
572 adsorption.

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Table 1. Mineralogical composition of assayed commercial products (C1–C7).

Commercial binder	Composition
C1	Bentonite (1 m558), Sepiolite (E-562)
C2	Processed montmorillonite, diatomaceous earth, yeast walls, seaweed extracts, sugar cane molasses
C3	Montmorillonite, interspersed montmorillonite, diatomaceous earth, yeast walls, seaweed extracts
C4	Bentonite (1 m558), strains of microorganisms (1m01 y 1m03), diatomaceous earth, E551 c, seaweed meal
C5	Bentonite (1 m558), calcium carbonate, yeast
C6	Di-octahedral bentonite
C7	Activated bentonite

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Table 2. ZEN adsorption percentages of adsorbents tested at 0.02 (w/v) versus six ZEN concentrations in gastric (pH 1.3) and intestinal (pH 6.8) simulated juices. B: bentonites; C: commercial adsorbents.

		Adsorption (%) mg ZEN/L					
Adsorbent	Juice	0.10	0.50	1	2	3	5
B1	Gastric	22.8	11.8	8.51	6.87	6.32	6.48
	Intestinal	30.9	10.1	6.31	4.83	4.58	4.16
B2	Gastric	30.8	11.3	8.02	5.65	4.53	4.44
	Intestinal	41.2	16.8	12.1	6.52	6.24	3.60
B3	Gastric	24.7	9.21	6.32	5.03	4.17	3.07
	Intestinal	41.7	11.2	6.49	5.38	5.03	3.11
B4	Gastric	47.2	11.9	9.22	7.88	6.93	6.50
	Intestinal	30.6	17.0	14.5	10.7	10.6	8.31
B5	Gastric	28.3	6.02	4.69	2.76	2.64	2.54
	Intestinal	32.3	8.27	6.75	5.46	4.59	3.98
B6	Gastric	39.1	10.9	9.51	6.87	6.19	6.18
	Intestinal	70.4	18.8	16.4	15.1	13.7	11.8
B7	Gastric	31.9	7.11	5.78	3.24	3.72	3.75
	Intestinal	49.4	12.5	11.6	8.87	8.47	7.36
C1	Gastric	32.9	8.00	5.97	3.69	2.97	2.17
	Intestinal	41.0	10.8	7.50	5.86	4.55	3.05
C2	Gastric	33.7	8.43	4.36	4.30	2.99	2.46
	Intestinal	35.8	17.1	10.7	7.49	5.69	3.58
C3	Gastric	42.1	11.7	9.79	8.14	6.71	6.28
	Intestinal	17.6	9.67	7.95	6.14	5.13	3.57
C4	Gastric	25.6	5.59	2.78	2.78	2.25	2.00
	Intestinal	52.7	7.01	6.89	6.52	4.13	2.96
C5	Gastric	25.1	4.29	3.55	3.11	2.51	2.08
	Intestinal	50.1	13.1	11.3	8.52	6.88	6.00
C6	Gastric	22.5	5.85	2.68	1.38	2.12	1.46
	Intestinal	21.1	5.83	4.05	2.08	1.20	1.30
C7	Gastric	22.8	6.68	4.16	2.50	1.52	1.89
	Intestinal	25.4	7.76	4.78	4.17	2.75	2.73

Values are average level of three triplicates. % RSDs in all cases were <10.

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Table 3. FB_1 adsorption percentages of adsorbents tested at 0.02 (w/v) versus six FB_1 concentrations in acid (pH 1.3) and basic (pH 6.8) buffer. B: bentonites; C: commercial adsorbents.

		Adsorption (%) mg FB1/L					
Adsorbent	Juice	1	2	4	6	8	10
B1	Gastric	>97.6	>97.6	>97.6	93.8	89.2	79.1
	Intestinal	1.93	3.47	3.64	2.84	4.17	<1.00
B2	Gastric	>95.3	>95.3	91.6	88.6	82.5	82.7
	Intestinal	5.30	4.86	4.63	5.70	5.89	5.87
B3	Gastric	72.5	69.1	55.3	49.0	44.4	36.7
	Intestinal	8.84	9.10	9.19	9.01	8.42	7.52
B4	Gastric	>89.1	87.2	86.8	80.4	72.5	65.9
	Intestinal	17.3	8.66	8.37	5.60	2.65	<1.00
B5	Gastric	>97.6	>97.6	>97.6	94.9	90.7	83.7
	Intestinal	5.80	4.85	6.32	5.84	5.55	4.44
B6	Gastric	>97.3	>97.3	>97.3	95.9	95.6	90.6
	Intestinal	5.03	6.06	7.34	6.86	5.33	5.36
B7	Gastric	>94.7	>94.7	91.5	90.2	77.6	69.6
	Intestinal	<1.00	1.87	2.80	3.70	3.10	3.31
C1	Gastric	>91.1	79.8	73.0	66.3	58.1	53.3
	Intestinal	6.91	5.44	5.82	3.28	5.37	5.05
C2	Gastric	28.1	13.20	10.39	11.71	6.86	11.92
	Intestinal	4.74	<1.00	<1.00	<1.00	<1.00	<1.00
C3	Gastric	1.18	2.36	2.76	4.05	5.44	5.51
	Intestinal	3.58	<1.00	3.18	3.23	3.46	5.28
C4	Gastric	64.8	67.2	65.6	60.3	53.3	50.0
	Intestinal	4.84	<1.00	4.44	1.45	<1.00	1.87
C5	Gastric	>94.9	>94.9	87.6	82.7	71.4	51.9
	Intestinal	4.95	6.01	8.52	7.19	7.43	7.60
C6	Gastric	35.6	27.9	13.1	10.3	5.81	<1.00
	Intestinal	4.75	5.59	5.17	3.52	3.74	2.71
C7	Gastric	34.2	33.8	28.5	23.3	19.3	8.73
	Intestinal	3.11	4.56	7.47	7.86	8.43	8.64

Values are average level of three triplicates. % RSDs in all cases were <10.

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Table 4. DON adsorption percentages of adsorbents tested at 0.02 (w/v) versus six DON concentrations in gastric (pH 1.3) and intestinal (pH 6.8) simulated juices. B: bentonites; C: commercial adsorbents.

		Adsorption (%) mg DON/L					
Adsorbent	Juice	2	4	6	8	10	12
B1	Gastric	8.39	7.84	8.34	6.07	8.42	8.22
	Intestinal	4.29	1.00	1.24	1.04	1.42	3.13
B2	Gastric	16.5	6.21	4.20	3.67	2.20	<1.00
	Intestinal	21.7	13.9	10.9	10.8	9.28	7.87
B3	Gastric	14.5	9.73	6.73	5.24	4.30	<1.00
	Intestinal	10.7	5.38	1.70	1.75	1.37	1.21
B4	Gastric	15.1	9.19	8.81	6.57	4.13	1.89
	Intestinal	10.6	6.56	3.20	2.89	1.71	<1.00
B5	Gastric	9.05	1.62	<1.00	<1.00	<1.00	<1.00
	Intestinal	8.35	2.07	1.65	1.54	1.19	2.11
B6	Gastric	7.19	3.59	2.78	1.89	1.74	1.28
	Intestinal	7.85	2.59	<1.00	<1.00	<1.00	<1.00
B7	Gastric	11.1	5.61	5.12	4.51	3.94	2.86
	Intestinal	18.3	4.69	2.86	2.99	4.58	3.03
C1	Gastric	14.3	10.1	6.46	4.45	2.97	1.49
	Intestinal	9.38	7.55	7.05	3.94	3.11	<1.00
C2	Gastric	12.3	7.65	3.57	1.45	1.31	1.30
	Intestinal	11.8	8.53	4.43	2.77	2.39	<1.00
C3	Gastric	7.50	5.97	4.94	4.73	4.32	2.61
	Intestinal	9.29	6.37	4.98	3.85	3.31	1.29
C4	Gastric	15.7	5.05	4.47	3.02	1.44	<1.00
	Intestinal	16.8	12.2	8.74	7.70	3.23	2.69
C5	Gastric	4.75	2.70	1.05	0.87	1.11	<1.00
	Intestinal	9.31	5.11	2.19	3.92	1.93	<1.00
C6	Gastric	18.1	6.29	2.43	<1.00	<1.00	<1.00
	Intestinal	12.1	1.03	<1.00	<1.00	<1.00	<1.00
C7	Gastric	4.27	5.94	5.01	3.96	5.70	4.24
	Intestinal	6.93	3.07	<1.00	<1.00	<1.00	<1.00

Values are average level of three triplicates. % RSDs in all cases were <10.

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Table 5. Langmuir and Freundlich parameters of 14 adsorbents tested at 0.02% (w/v), in gastric (pH 1.3) and intestinal (pH 6.8) simulated juice with ZEN (0.1–5 mg/L). B: bentonites; C: commercial adsorbents.

Adsorbent	Juice	Q_{max}(mg/g)	K_L(L/mg)	RMSE	K_F(mg/L)	n	RMSE
B1	Gastric	10.1 ± 8.2	0.04 ± 0.1	0.1060	0.47 ± 0.04	1.20 ± 0.1	0.0843
	Intestinal	2.22 ± 1.0	0.17 ± 0.1	0.0829	0.35 ± 0.03	1.50 ± 0.2	0.0596
B2	Gastric	2.03 ± 0.7	0.24 ± 0.1	0.1126	0.43 ± 0.04	1.68 ± 0.2	0.0720
	Intestinal	0.77 ± 0.1	3.60 ± 1.7	0.0861	0.53 ± 0.02	3.63 ± 0.6	0.0603
B3	Gastric	1.12 ± 0.1	0.50 ± 0.1	0.0406	0.37 ± 0.01	2.01 ± 0.1	0.0225
	Intestinal	0.75 ± 0.2	0.96 ± 0.7	0.0962	0.35 ± 0.03	2.52 ± 0.5	0.0660
B4	Gastric	4.55 ± 1.9	0.12 ± 0.1	0.1200	0.56 ± 0.05	1.39 ± 0.1	0.0902
	Intestinal	3.54 ± 0.9	0.28 ± 0.1	0.1347	0.80 ± 0.04	1.59 ± 0.1	0.0731
B5	Gastric	1.39 ± 0.8	0.16 ± 0.1	0.0717	0.22 ± 0.03	1.58 ± 0.3	0.0543
	Intestinal	1.65 ± 0.4	0.27 ± 0.1	0.0591	0.36 ± 0.02	1.61 ± 0.1	0.0368
B6	Gastric	6.24 ± 4.0	0.07 ± 0.1	0.0854	0.45 ± 0.03	1.23 ± 0.1	0.0676
	Intestinal	5.69 ± 0.8	0.24 ± 0.1	0.0793	1.12 ± 0.03	1.42 ± 0.1	0.0679
B7	Gastric	7.05 ± 15	0.03 ± 0.1	0.0919	0.25 ± 0.04	1.21 ± 0.2	0.0842
	Intestinal	3.70 ± 0.9	0.20 ± 0.1	0.0797	0.64 ± 0.02	1.43 ± 0.1	0.0526
C1	Gastric	0.59 ± 0.1	1.16 ± 0.6	0.0622	0.30 ± 0.01	2.85 ± 0.2	0.0246
	Intestinal	0.84 ± 0.1	1.07 ± 0.4	0.0681	0.41 ± 0.02	2.67 ± 0.3	0.0428
C2	Gastric	0.81 ± 0.2	0.56 ± 0.4	0.0829	0.30 ± 0.02	2.30 ± 0.4	0.0565
	Intestinal	0.44 ± 0.1	2.25 ± 1.3	0.0582	0.28 ± 0.01	3.70 ± 0.5	0.0259
C3	Gastric	3.36 ± 1.0	0.19 ± 0.1	0.1049	0.56 ± 0.04	1.48 ± 0.1	0.0712
	Intestinal	1.36 ± 0.1	0.53 ± 0.1	0.0371	0.46 ± 0.02	2.04 ± 0.2	0.0488
C4	Gastric	0.92 ± 0.5	0.22 ± 0.2	0.0662	0.19 ± 0.02	1.76 ± 0.4	0.0535
	Intestinal	0.96 ± 0.2	0.73 ± 0.4	0.0841	0.39 ± 0.04	2.32 ± 0.6	0.0929
C5	Gastric	0.88 ± 0.2	0.28 ± 0.1	0.0380	0.20 ± 0.01	1.67 ± 0.2	0.0289
	Intestinal	2.17 ± 0.4	0.38 ± 0.1	0.0912	0.61 ± 0.02	1.75 ± 0.1	0.0471
C6	Gastric	0.62 ± 0.4	0.29 ± 0.3	0.0766	0.16 ± 0.03	2.08 ± 0.7	0.0633
	Intestinal	0.23 ± 0.0	5.74 ± 5.9	0.0539	0.17 ± 0.01	3.98 ± 1.4	0.0391
C7	Gastric	0.52 ± 0.2	0.68 ± 0.7	0.0921	0.21 ± 0.03	2.41 ± 0.8	0.0691
	Intestinal	0.92 ± 0.2	0.22 ± 0.2	0.0662	0.27 ± 0.02	1.90 ± 0.4	0.0534

Q_{max}: maximum adsorption capacity; K_L: Langmuir affinity constant; K_F: Freundlich capacity constant; n: adsorption intensity; RMSE: root-mean-square error.

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Table 6. Langmuir and Freundlich parameters of 14 adsorbents tested at 0.02% (w/v), in acid (pH 1.3) and in basic (pH 6.8) buffer with FB_1 (1–10 mg/L). B: bentonites; C: commercial adsorbents.

Adsorbent	Juice	Q_{max} (mg/g)	K_L(L/mg)	RMSE	K_F(mg/L)	n	RMSE
B1	Gastric	34.9 ± 5.2	4.76 ± 2.1	4.0521	27.8 ± 3.3	2.63 ± 0.7	5.1128
	Intestinal	NC	NC	NC	NC	NC	NC
B2	Gastric	41.6 ± 5.4	1.94 ± 0.6	2.2036	26.8 ± 1.2	1.88 ± 0.2	2.2323
	Intestinal	NC	NC	NC	0.24 ± 0.1	0.89 ± 0.1	0.1187
B3	Gastric	15.9 ± 0.9	0.86 ± 0.1	0.5036	6.86 ± 0.4	2.20 ± 0.2	0.7178
	Intestinal	12.4 ± 3.4	0.05 ± 0.0	0.1508	0.65 ± 0.1	1.21 ± 0.1	0.2054
B4	Gastric	26.1 ± 1.0	1.95 ± 0.2	0.6146	15.8 ± 0.8	2.22 ± 0.3	1.7650
	Intestinal	NC	NC	NC	NC	NC	NC
B5	Gastric	37.9 ± 6.3	4.54 ± 2.1	4.2283	31.4 ± 4.0	2.39 ± 0.6	5.2265
	Intestinal	NC	NC	NC	0.46 ± 0.1	1.41 ± 0.4	0.3113
B6	Gastric	47.9 ± 13.6	3.46 ± 1.9	4.5434	43.8 ± 9.1	1.79 ± 0.5	5.4625
	Intestinal	NC	NC	NC	0.46 ± 0.1	1.24 ± 0.2	0.2613
B7	Gastric	26.9 ± 2.3	3.62 ± 0.9	1.8855	19.4 ± 1.6	2.61 ± 0.6	3.1009
	Intestinal	NC	NC	NC	0.17 ± 0.1	1.03 ± 0.3	0.2387
C1	Gastric	25.9 ± 2.0	0.02 ± 0.1	1.0414	11.9 ± 0.4	2.24 ± 0.1	0.7429
	Intestinal	NC	NC	NC	0.27 ± 0.1	1.01 ± 0.2	0.1776
C2	Gastric	NC	NC	NC	0.85 ± 0.5	1.29 ± 0.5	0.8201
	Intestinal	NC	NC	NC	NC	NC	NC
C3	Gastric	NC	NC	NC	0.10 ± 0.0	0.62 ± 0.1	0.0967
	Intestinal	NC	NC	NC	0.04 ± 0.0	0.53 ± 0.1	0.1826
C4	Gastric	26.3 ± 2.0	0.50 ± 0.1	0.5170	8.28 ± 0.5	1.64 ± 0.2	1.0445
	Intestinal	NC	NC	NC	NC	NC	NC
C5	Gastric	21.8 ± 1.8	4.57 ± 1.5	1.8970	15.8 ± 1.6	3.54 ± 1.1	3.6139
	Intestinal	NC	NC	NC	0.56 ± 0.2	1.31 ± 0.3	0.4321
C6	Gastric	NC	NC	NC	NC	NC	NC
	Intestinal	NC	NC	NC	0.52 ± 0.3	2.58 ± 1.9	0.4198
C7	Gastric	NC	NC	NC	3.90 ± 1.3	3.25 ± 2.1	2.1499
	Intestinal	NC	NC	NC	0.38 ± 0.1	0.95 ± 0.1	0.4180

Q_{max}: maximum adsorption capacity; K_L: Langmuir affinity constant; K_F: Freundlich capacity constant; n: adsorption intensity; RMSE: root-mean-square error; NC: not converge.

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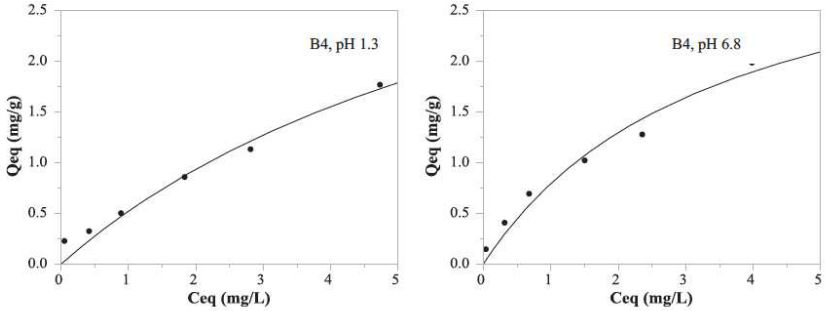

Figure 1. Langmuir isotherms obtained at 37°C in gastric ((a) at pH 1.3) and intestinal ((b) at pH 6.8) simulated juices by testing a fixed amount of adsorbent B4 (0.02%, w/v) versus increasing concentrations of ZEN (0.1–5 mg/L).

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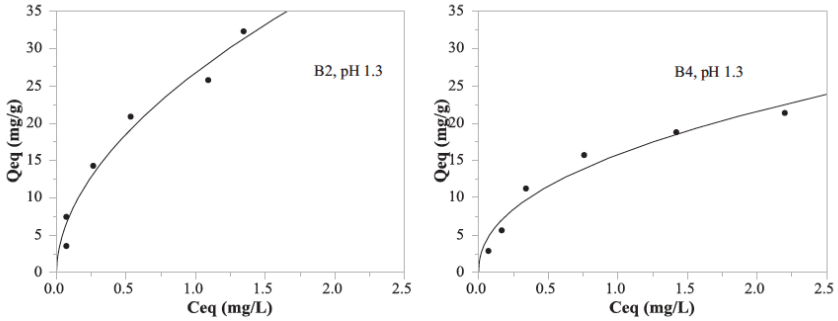

Figure 2. Freundlich isotherms obtained at 37°C in an acid buffer (at pH 1.3) by testing a fixed amount of adsorbent B2 (a) and B4 (b) (0.02%, w/v) versus increasing concentrations of FB_1 (1–10 mg/L).

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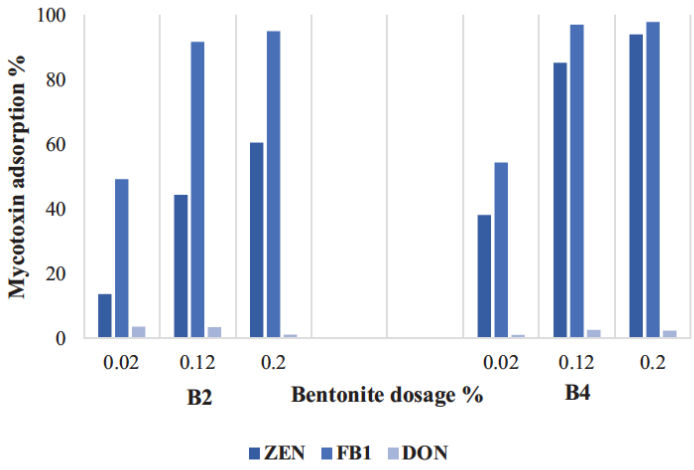

Figure 3. Effect of B2 (a) and B4 (b) adsorbent dosage on FB_1, ZEN, and DON adsorption.

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